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(54) Title: POLYPEPTIDES AND PHARMACEUTICAL COMPOSITIONS COMPRISING THE SAME FOR THE PREVENTION AND TREATMENT OF COMPLICATIONS ASSOCIATED WITH INFECTIOUS DISEASES

(57) Abstract: The present invention relates to polypeptides and pharmaceutical preparations that can be used in the prevention and treatment of complications associated with infectious diseases.

Polypeptides and pharmaceutical compositions comprising the same for the prevention and treatment of complications associated with infectious diseases

The present invention relates to polypeptides and pharmaceutical preparations that can be used in the prevention and treatment of complications associated with infectious diseases.

Infectious diseases give rise to complications that are caused by an activation of the coagulation cascade, varying from subclinical activation (which is indicated by a rise in laboratory markers for thrombin and fibrin generation) to severe thrombocytopenia, or even thrombotic thrombocytopenic purpura (TTP) or disseminated intravascular coagulation (DIC) (Levi et al., JAMA, 270: 975-979 (1993)). Vascular endothelial injury, caused by the pathogen, is considered as one of the primary events in these complications, which, as shown in recent studies, may lead to excessive release of extremely large polymers of von Willebrand Factor (vWF) that cannot be processed to smaller forms because of an insufficient availability of vWF-cleaving metalloprotease. Recent data led to the hypothesis that patients with acute, sporadic thrombocytopenic disorders (e.g., occurring as complications associated with infectious diseases) have antibody-mediated inhibition of the plasma metalloprotease (Tsai et al., N Engl J Med, 339: 1585-1594 (1998); Mannucci et al., Blood, 74: 978-983 (1989)). Next to bacterial pathogens, activation of the coagulation system via this mechanism has been documented for viruses causing hemorrhagic fevers (HFs) (Bhamarapravati et al., Rev Infect Dis, 11(suppl): S826-829 (1989); Heller et al., Thromb Haemost, 73: 368-373 (1995)), protozoa (such as the intraerythrocytic protozoa of the genus *Plasmodium* causing malaria) (Clemens et al., Br J Haematol, 87: 100-105 (1994); Mohanty et al., Am J Haematol, 54: 23-29 (1997)), and fungi (Fera et al., Infection, 21: 171-173 (1993)). Since no specific treatment is yet available for thrombocytopenic disorders such as TTP or DIC, therapy focuses on the treatment of the underlying disease (e.g., antibiotics for bacterial infection). However, for many infectious diseases, such as viral HF, causal therapy is not available, and only supportive care can be provided.

Accordingly, there is a need for novel therapeutic agents that can be used in the prevention and treatment of complications associated with infectious diseases.

It has now been found that polypeptides directed against vWF and pharmaceutical compositions comprising the same can be used in the prevention and treatment of complications associated with infectious diseases.

More in particular, it has been found that polypeptides comprising or essentially consisting of at least one Nanobody® directed against vWF and pharmaceutical compositions comprising the same can be used in the prevention and treatment of complications associated with infectious diseases.

Such Nanobodies™ and polypeptides are for example described in WO 04062551 and non-prepublished co-pending US provisional application 60/683,474 entitled 'Improved Nanobodies™ for the treatment of aggregation-mediated disorders' (filing date: May 20th 2005).

The present invention therefore relates to the use of polypeptides directed against vWF for the preparation of a medicament for the prevention and/or treatment of complications associated with and/or caused by infectious diseases.

More specifically, the present invention relates to the use of polypeptides which competitively inhibit the interaction of vWF to gpIb for the preparation of a medicament for the prevention and/or treatment of complications associated with and/or caused by infectious diseases.

Preferably the polypeptides used in the present invention comprise or essentially consist of at least one immunoglobulin sequence or immunoglobulin fragment. Examples of such immunoglobulin sequences or immunoglobulin fragments are Fab fragments, F(ab') fragments, $F(ab_2)$ fragments, Fv fragments, scFv fragments.

More preferably, the polypeptides used in the present invention comprise or essentially consist of at least one immunoglobulin variable domain.

Still more preferably, the polypeptides used in the present invention comprise or essentially consist of at least one single domain antibody.

Most preferably, the polypeptides used in the present invention comprise or essentially consist of at least one Nanobody®.

Polypeptides that are specifically preferred for use in the present invention are described in WO04062551 and non-prepublished co-pending US provisional application 60/683,474 entitled 'Improved Nanobodies™ for the treatment of aggregation-mediated disorders' (filing date: May 20th 2005).

More preferred for use in the present invention are polypeptides directed against the A1 domain of vWF and in particular the A1 domain of activated vWF and/or the A3 domain of vWF as described in WO 04062551 and in non-prepublished co-pending US provisional application 60/683,474 entitled 'Improved Nanobodies™ for the treatment of aggregation-mediated disorders' (filing date: May 20th 2005).

Most preferred for use in the present invention are Nanobodies™ directed against vWF as described in non-prepublished co-pending US provisional application 60/683,474 entitled 'Improved Nanobodies™ for the treatment of aggregation-mediated disorders' (filing date: May 20th 2005).

The present invention furthermore relates to the use of pharmaceutical preparations comprising at least one polypeptide as described above for the prevention and/or treatment of complications associated with and/or caused by infectious diseases. Such pharmaceutical compositions may for example be as described in WO 04062551 and non-prepublished co-pending US provisional application 60/683,474 entitled 'Improved Nanobodies™ for the treatment of aggregation-mediated disorders' (filing date: May 20th 2005).

Polypeptides, pharmaceutical compositions, and uses that are preferred according WO 04062551 and in the non-prepublished co-pending US provisional application 60/683,474 entitled 'Improved Nanobodies™ for the treatment of aggregation-mediated disorders' (filing date: May 20th 2005) are also preferred for use in the present invention. In particular, polypeptides and pharmaceutical compositions comprising bivalent Nanobodies™ as described in WO 04062551 and in the non-prepublished co-pending US provisional application 60/683,474 entitled 'Improved Nanobodies™ for the treatment of aggregation-mediated disorders' (filing date: May 20th 2005) may be used in the present invention.

Polypeptides and pharmaceutical preparations of the present invention generally can be used in the prevention and treatment of the complications of infectious diseases.

Such infectious diseases include but are not limited to sepsis, hemorrhagic fevers, malaria, AIDS, endotoxemia, leptospirosis, gastroenteritis, rheumatoid arthritis, viral diarrheas, viral pneumonia, influenza, hepatitis, viral leukemia, herpes, cytomegalovirus infection, infectious mononucleosis, and other diseases caused by bacterial or non-bacterial infectious pathogens, as will be clear to the skilled person. Complications of such

infectious diseases that can be prevented or treated using the methods of the present invention include but are not limited to thrombocytopenia, disseminated intravascular coagulation (DIC), hemolytic uremic syndrome (HUS), thrombotic thrombocytopenic purpura (TTP), vasculitis, and other thrombohemorrhagic complications or syndromes, as will again be clear to the skilled person.

Reference is for example made to the prior art mentioned above and to the further references cited therein.

EXAMPLES

Example 1: Clinical course of healthy humans after exposure to *P. falciparum*-infected mosquitoes and determination of platelet number

After fourteen healthy humans were exposed to *P. falciparum*-infected mosquitoes, they all became parasitemic. Figure 1 shows that the quantitative nucleic acid sequence-based amplification (QT-NASBA; Schoone, G. J., L. Oskam, N. C. M. Kroon, H. D. F. H. Schallig, and S. A. Omar. 2000. Detection and quantification of *Plasmodium falciparum* in blood samples using quantitative nucleic acid sequence-based amplification. J. Clin. Microbiol. **38**:4072-4075) became positive after a median time of 7.0 days (range 6.0 to 9.0 days) post-infection and showed increasing parasitemia before the initiation of antimalarial treatment. Treatment was started after a median time of 9.65 days (range 7.3 to 11.3) immediately upon microscopic detection of *P. falciparum* parasites in a thick blood smear. Figure 2 shows a decline in platelet count almost instantly after the onset of blood-stage infection and reached a nadir of 58.6% (95%CI 46.8-70.4%) of baseline level. Successful antimalarial treatment was followed by a recovery of the platelet count to a maximum mean value of 142% (95%CI 130.2-154.4%) of baseline level at day 21 post-infection before returning to baseline level at day 42. Hemoglobin levels remained unchanged throughout the infection.

Example 2: Time course of vWF, vWF-propeptide and active vWF

Figure 3A shows the time course of vWF in the fourteen *P. falciparum*-infected human patients. vWF levels started to increase almost immediately after onset of blood-stage infection and reached a mean peak level of 190% above baseline. The range of

individual peak levels was 115 to 385% above baseline. vWF-propeptide followed a similar kinetic pattern with a maximum mean peak level of 238% above baseline and with individual peak levels ranging from 129 to 291% above baseline (Figure 3B). With the use of the Nanobody® AU/VWF-a11, which specifically recognizes the GpIb-binding conformation of the vWF A1 domain, the amounts of active vWF were determined at three time points during the infection: (1) baseline, (2) the day with the first significant decrease in platelet count, defined as a decrease of at least $20x10^9$ platelets/liter compared to the previous day, and (3) the day with the lowest platelet count. As shown in figure 4, when the baseline level was set to be 1 for each patient, the mean relative vWF activation factor was 1.5 (95% CI 1.0-2.0) at the day of the first decrease in platelets and 2.3 (95% CI 1.2-3.3) at the day of platelet count nadir.

Example 3: ADAMTS-13 activity

To determine whether a decrease in ADAMTS-13 activity could account for the increased levels of (active) vWF, we quantitatively measured this ADAMTS-13 activity at baseline and at day 8 post-infection in the fourteen *P. falciparum*-infected human patients. The mean ADAMTS-13 activity at day 8 postinfection was 99.9% (range 83-127%) of baseline activity. Therefore, the elevated levels of (active) vWF could not be explained by decreased proteolysis of large vWF multimers.

Example 4: Relation between (active) vWF, platelet count and parasitemia

To examine the relationship between platelet count, vWF and vWF activation factor, we plotted the relative values of these variables against each other. A strong inverse relationship (Pearson r= -0.62, $p<0.0001$) was present between vWF levels and platelet counts during the course of the infection (figure 5A). A similar strong association (Pearson's r=-0.61, p=0.021) was found between vWF levels and platelet counts at the day of platelet count nadir (figure 5B). Moreover, vWF activation factor at that day related significantly (Pearson r=-0.58; p=0.03) to the relative platelet count as well (figure 5C). The degree of parasitemia (expressed as log number of parasites per milliliter blood) from the onset of parasitemia until the start of antimalarial treatment, correlated weakly (Pearson r= 0.3741; p=0.0035) with corresponding relative vWF levels.

Example 5: Time course of cytokines and C-reactive protein

Figure 6 compares the kinetics of relative vWF levels with plasma CRP and interleukin (IL)-1ra levels, both representative markers of inflammation. The initial increase in vWF was not preceded by an increase in either CRP or IL-1ra. The pro-inflammatory cytokines tumor necrosis factor-α and IL-1ra did not show a significant increment throughout the infection.

FIGURES

Figure 1: Kinetics of *P. falciparum* parasitemia before anti-malarial treatment. 14 healthy humans were experimentally infected with *P. falciparum*. Data are mean (+/- SEM) parasitemia before antimalarial treatment as determined by QT-NASBA. The numbers of humans tested at indicated time points are included between brackets.

Figure 2: Kinetics of platelet count in P. falciparum blood-stage infection. 14 healthy humans were experimentally infected with *P. falciparum*. Data are the mean (+/- SEM) of relative (% of baseline) platelet counts during blood stage infection (* mean of 10 humans; ** mean of 8 humans). The mean absolute platelet count at baseline was $249x10^9$ platelets/liter (95% CI 222-$276x10^9$). Changes in relative platelet counts throughout the infection were significant ($p<0.0001$; repeated measures ANOVA).

Figure 3: Kinetics of vWF and vWF-propeptide in *P. falciparum* blood-stage infection. 14 healthy humans were experimentally infected with *P. falciparum*. Data presented are the mean (+/- SEM) of relative levels of vWF (a) and vWF-propeptide (b) during the blood-stage infection until 2 days after initiation of antimalarial treatment (* mean of 11 humans; ** mean of 4 humans). Mean absolute baseline levels of vWF and propeptide were 35.3 nM (95%CI 26.5-44.0) and 5.8nM (95%CI 5.1-6.6), respectively. Changes in both vWF and vWF propeptide during the course of the infection were significant ($p<0.0001$; repeated measures ANOVA).

Figure 4: vWF-activation factor in *P. falciparum* blood-stage infection. 14 healthy humans were experimentally infected with *P. falciparum.* Data are the vWF activation factors at three time points: 1) baseline, (2) the day with the first significant decrease in platelet count (>$20x10^9$ platelets/liter), and (3) the day of platelet count nadir. The activation factor at baseline was set to be 1 for each human (Repeated measures ANOVA).

Figure 5: Correlations between platelet count, vWF and vWF-activation factor. 14 healthy humans were experimentally infected with *P. falciparum.* (A) Daily vWF levels against corresponding platelet counts during blood-stage infection; (B) vWF levels against platelet counts at the day of platelet count nadir; (C) vWF activation factors against platelet counts at the day of platelet count nadir.

Figure 6: Comparisons between kinetics of CRP, IL-1ra, and vWF. 14 healthy humans were experimentally infected with *P. falciparum.* Data represent the mean (+/-SEM) of plasma levels of CRP (A) and IL-1ra (B) plotted against the mean (+/-SEM) of relevant vWF levels during the first days of blood-stage infection.

CLAIMS

1. Use of a polypeptide directed against von Willebrand Factor (vWF) for the preparation of a medicament for the prevention or treatment of complications associated with infectious diseases.

2. Use of a polypeptide according to claim 1, wherein said polypeptide competitively inhibits the interaction of vWF to gpIb.

3. Use of a polypeptide according to claim 1 or 2, wherein said polypeptide is directed against the A1 domain of activated vWF and/or the A3 domain of vWF.

4. Use of a polypeptide according to any of claims 1 to 3, wherein said polypeptide comprises or essentially consists of at least one immunoglobulin sequence or immunoglobulin fragment

5. Use of a polypeptide according to any of claims 1 to 4, wherein said polypeptide comprises or essentially consists of at least one immunoglobulin variable domain.

6. Use of a polypeptide according to any of claims 1 to 5, wherein said polypeptide comprises or essentially consists of at least one single domain antibody.

7. Use of a polypeptide according to any of claims 1 to 6, wherein said polypeptide comprises or essentially consists of at least one Nanobody®.

8. Use of a polypeptide according to any of claims 1 to 7, wherein said complications are chosen from the group consisting of thrombohemorrhagic complications.

9. Use of a polypeptide according to claim 8, wherein said thrombohemorrhagic complications are chosen from the group consisting of thrombocytopenia,

disseminated intravascular coagulation (DIC), hemolytic uremic syndrome (HUS), thrombotic thrombocytopenic purpura (TTP), or vasculitis.

10. Use of a polypeptide according to any of claims 1 to 9, wherein said infectious diseases are chosen from the group consisting of diseases caused by bacterial or non-bacterial infectious pathogens.

11. Use of a polypeptide according to claim 10, wherein said infectious diseases are chosen from the group consisting of sepsis, hemorrhagic fevers, malaria, AIDS, endotoxemia, leptospirosis, gastroenteritis, rheumatoid arthritis, viral diarrheas, viral pneumonia, influenza, hepatitis, viral leukemia, herpes, cytomegalovirus infection, infectious mononucleosis.

Figure 1

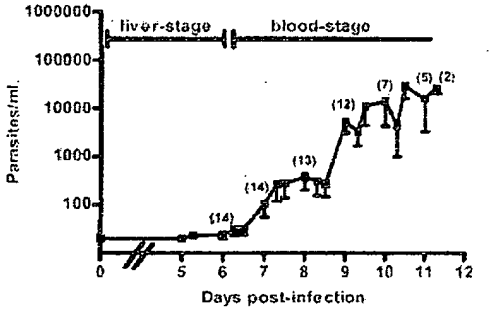

Figure 2

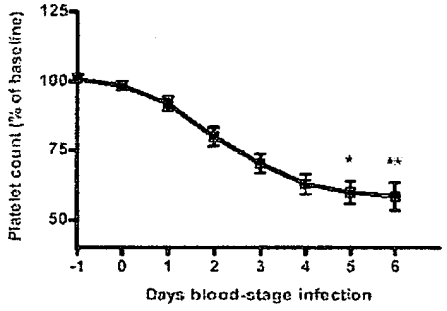

Figure 3

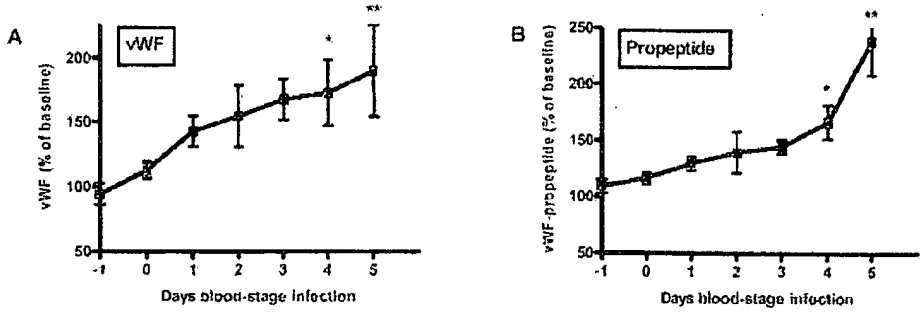

Figure 4

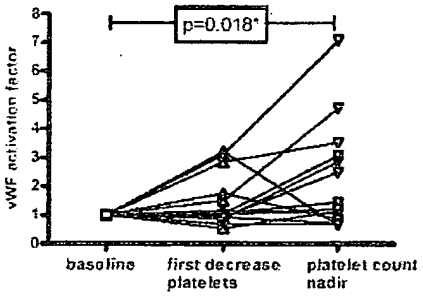

Figure 5

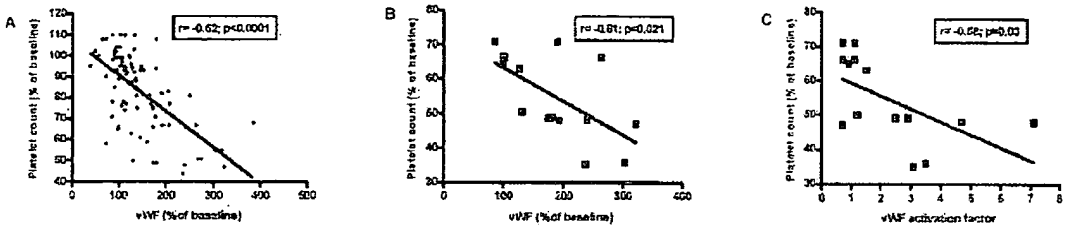

Figure 6